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(FILE 'HOME' ENTERED AT 08:54:14 ON 08 MAY 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,  
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,  
CABA,  
CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,  
DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 08:54:26 ON  
08 MAY 2002

SEA GDP-MANNOSE

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48 FILE AGRICOLA  
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5 FILE BIOBUSINESS  
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931 FILE CAPLUS  
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L1

QUE GDP-MANNOSE  
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FILE 'CAPLUS, BIOSIS, MEDLINE, EMBASE, SCISEARCH, BIOTECHNO' ENTERED AT  
08:56:22 ON 08 MAY 2002

L2 1874 S L1 AND (SYNTHESI? OR BIOSYNTHESI OR PRODUC? OR MANUFACTUR?)  
L3 96 S L2 AND (HYBRID OR CHIMER? OR FUSION OR DUAL-FUNCTION)  
L4 32 DUP REM L3 (64 DUPLICATES REMOVED)

4 ANSWER 16 OF 32 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6  
ACCESSION NUMBER: 1999:37915 CAPLUS  
DOCUMENT NUMBER: 130:193578  
TITLE: GDP-fucose synthetase from Escherichia coli:  
structure

of a unique member of the short-chain  
dehydrogenase/reductase family that catalyzes two  
distinct reactions at the same active site  
AUTHOR(S): Somers, William S.; Stahl, Mark L.; Sullivan, Francis  
X.  
CORPORATE SOURCE: Small Molecule Drug Discovery, Genetics Institute,  
Inc., Cambridge, MA, 02140, USA  
SOURCE: Structure (London) (1998), 6(12), 1601-1612  
CODEN: STRUE6; ISSN: 0969-2126  
PUBLISHER: Current Biology Publications  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB In all species examd., GDP-fucose is **synthesized** from  
GDP-mannose in a 3-step reaction catalyzed by 2 enzymes,  
GDP-mannose 4,6-dehydratase and a **dual**  
function 3,5-epimerase-4-reductase named GDP-fucose synthetase  
(I). In this latter aspect, fucose **biosynthesis** differs from  
that of other deoxy and dideoxy sugars, in which the epimerase and  
reductase activities are present as sep. enzymes. Defects in GDP-fucose  
**biosynthesis** have been shown to affect nodulation in bacteria,  
stem development in plants, and are assocd. with the immune defect  
leukocyte adhesion deficiency type II in humans. Here, the authors detd.  
the structure of I from E. coli at 2.2 Å. The structure of I  
was found to be closely related to that of UDP-galactose 4-epimerase, and  
more distantly to other members of the short-chain  
dehydrogenase/reductase

family. The authors also detd. the structures of binary complexes of I  
with its substrate, NADPH, and its **product**, NADP. The  
nicotinamide cofactors bound in the syn and anti conformations, resp. I  
bound its substrate, NADPH, in the proper orientation (syn) for  
transferring the 4-pro-S hydride of the nicotinamide. The authors obsd.

a single binding site in I for the second substrate, GDP-4-keto-6-deoxy-  
mannose. This implies that both the epimerization and redn. reactions  
occur at the same site in the enzyme. As is the case for all members of  
the short-chain family of dehydrogenase/reductases, I retained the  
Ser-Tyr-Lys catalytic triad. It is proposed that this catalytic triad  
functions in a mechanistically equiv. manner in both the epimerization  
and

redn. reactions. Addnl., the x-ray structure allowed the authors to  
identify other residues that were potentially required for substrate  
binding and catalysis.

L4 ANSWER 15 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1998:226493 BIOSIS

DOCUMENT NUMBER: PREV199800226493

TITLE: Molecular cloning of human **GDP-mannose**  
4,6-dehydratase and reconstitution of GDP-fucose  
**biosynthesis** in vitro.

AUTHOR(S): Sullivan, Francis X. (1); Kumar, Ravindra; Kriz, Ronald;  
Stahl, Mark; Xu, Guang-Yi; Rouse, Jason; Chang, Xiao-Jia;  
Boodhoo, Amechand; Potvin, Barry; Cumming, Dale A.

CORPORATE SOURCE: (1) Small Mol. Drug Discovery, Genet. Inst. Inc., 87  
Cambridgepark Dr., Cambridge, MA 02140 USA

SOURCE: Journal of Biological Chemistry, (April 3, 1998) Vol. 273,  
No. 14, pp. 8193-8202.  
ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

AB We have cloned the cDNA encoding human **GDP-mannose**  
4,6-dehydratase, the first enzyme in the pathway converting **GDP-**  
**mannose** to GDP-fucose. The message is expressed in all tissues and  
cell lines examined, and the cDNA complements Lec13, a Chinese Hamster  
Ovary cell line deficient in **GDP-mannose**  
4,6-dehydratase activity. The human **GDP-mannose**  
4,6-dehydratase polypeptide shares 61% identity with the enzyme from  
Escherichia coli, suggesting broad evolutionary conservation. Purified  
recombinant enzyme utilizes NADP+ as a cofactor and, like its E. coli  
counterpart, is inhibited by GDP-fucose, suggesting that this aspect of  
regulation is also conserved. We have isolated the **product** of  
the dehydratase reaction, GDP-4-keto-6-deoxymannose, and confirmed its  
structure by electrospray ionization-mass spectrometry and high field

NMR. Using purified recombinant human **GDP-mannose**  
4,6-dehydratase and FX protein (GDP-keto-6-deoxymannose 3,5-epimerase,  
4-reductase), we show that the two proteins alone are sufficient to  
convert **GDP-mannose** to GDP-fucose in vitro. This  
unequivocally demonstrates that the epimerase and reductase activities  
are  
on a single polypeptide. Finally, we show that the two homologous enzymes  
from E. coli are sufficient to carry out the same enzymatic pathway in